

**UNITED STATES DISTRICT COURT
DISTRICT OF MINNESOTA**

Animal Legal Defense Fund; and
Lockwood Animal Rescue Center,

Case No. 17-cv-4496

Judge Joan N. Ericksen

Plaintiffs,

v.

Fur-Ever Wild; Wolves, Woods &
Wildlife; and Teresa Lynn Petter,

Defendants.

Report on canid DNA tests for Animal Legal Defense Fund

Bridgett vonHoldt, Ph.D.

Assistant Professor of Ecology & Evolutionary Biology

Princeton University

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Qualifications and details of Dr. Bridgett vonHoldt.

I have been contracted by Animal Legal Defense Fund to review and report on the genetic testing results for an hourly fee of \$150. I have made no previous testimony.

Appointments

- **Assistant Professor** in Ecology & Evolutionary Biology at Princeton University (7/2013-present)
- **Post-doctoral scholar** of the Computational Biosciences Institute Fellowship at University of California, Los Angeles (2012-2013); Advisors: Robert Wayne & Matteo Pellegrini
- **Post-doctoral scholar** of computational genomics at University of California, Irvine (2010-2012); Advisor: Brandon Gaut

Education

- **Ph.D.** in Ecology & Evolutionary Biology at University of California, Los Angeles (2005-2010); Advisor: Robert K. Wayne
- **M.S.** in Biology at New York University (2002-2004); Advisor: Richard Borowsky
- **B.S.** in Psychology at Eckerd College (1998-2002); Advisor: Mark Davis

For an exhaustive list of my experience and expertise, please see my full CV, the last entry in this document.

Executive summary of the DNA evidence.

DNA results of the defendants' animals indicate that they all carry the majority, if not exclusively, gray wolf ancestry. Morphologic evidence of the animals also display characteristics consistent with gray wolves, although morphologic evidence is helpful but not sufficient for inferring species membership. Due to their shared and recent evolutionary history, dogs and wolves share a tremendous amount of genetic and morphological features. The father of each canine in question was a gray wolf, with an exceedingly low probability that the father was a dog. The genetics used to test the species identification of the mother was not informative. My interpretation of the nuclear genome and chromosomal patterns of ancestry is that there is no evidence that nine of the 10 canines tested are F1 hybrids. Nine of 10 canines tested were found to carry only negligible amounts of dog ancestry. The last canine (case #10) contained a non-negligible proportion of dog ancestry, which is possibly indicative of recent admixture with dog for this animal.

Summary of documents reviewed and considered.

Below is a summary of the evidence I reviewed. My interpretation of the documents is guided by my own publication record on genetics canine ancestry genetics (see my publications listed in my CV).

- (1) All DNA evidence in this case, including:
 - a. Evidence the plaintiffs obtained from Embark, regarding nine of the defendants' wolves (believed to be named Churakawa/Chiracowa, Crow, Sioux, Todd, Nahani, Tatonka, Wounded Knee, George, and Texas Red).
 - b. Evidence the plaintiffs obtained from UC Davis Veterinary Genetics Lab

- regarding 10 of the defendants' wolves (the nine above, plus a wolf believed to be named Wokon/Waukon)
- c. Evidence the defendants obtained from Embark, regarding six of the defendants' wolves (believed to be named Churakawa/Chiracowa, Crow, Sioux, Todd, Wokon/Waukon, Nahani).
- (2) I have corresponded via email with Dr. Adam Boyko from Embark and Dr. Robert Grahn of UC Davis Veterinary Genetics Laboratory. I inquired about the development and nature of their respective genetics tests. I have received information from both.
 - (3) All deposition transcripts in this case and all associated deposition exhibits, excluding attorneys' eyes only information.
 - (4) Images of wolves and wolf pelts contained in the following documents
 - a. ECF Doc 20.4 and ECF Doc 20-3, filed on the docket in this case.
 - b. Deposition Exhibits 14–20, 56–57, 59–61, 65–66, and 69–71.

Past experience with the DNA testing companies, Embark and UC Davis.

I have previously collaborated with and published scientific peer-reviewed papers with scientists employed by Embark or UC Davis's School of Veterinary Medicine. These publications were a result of a multi-institutional collaboration on canine genomics. They are highly qualified individuals and institutions with regard to canine genetics.

Defining terms used in this document.

The terms "*hybrid*" and "*admixed*" are ubiquitous in both the scientific and non-scientific community. Hybridization typically is used to describe the mating between two dissimilar "types", where "types" can refer to species, distinct genetic groups, breeds, subspecies, ecotypes, etc. A "hybrid" is the first-generation (F1) offspring produced by the mating between a wolf and a dog, or their hybridization. However, often the F1 offspring is restricted in terms of mates when it sexually matures and hypothetically has three options for mates: 1) mate with an unrelated wolf, 2) mate with an unrelated dog, or 3) mate with an F1 hybrid or another mixed-ancestry individual. In the first two instances, these are called "backcrosses" and will result in an admixed offspring with higher ancestry of that parent's taxonomic affiliation. The third instance also produces an "admixed" offspring but, accordingly to Mendel's laws of transmission genetics, these offspring will have more variable ancestry compositions across their genomes. An F1 hybrid has a very distinct and predictable profile of ancestry across its genome. As an F1 genome has 50% representation from each parent (due to each of their donations of a chromosomal copy), then the F1 hybrid genome is expected to appear as the schematic found in [Figure 1](#).

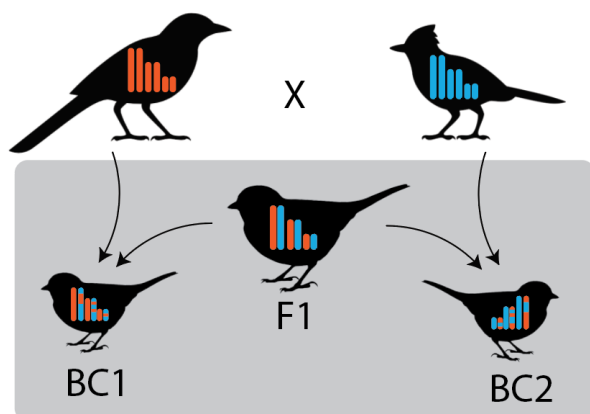


Figure 1. Cartoon of expected ancestry proportions in three admixed genomes: 1) a first-generation (F1) hybrid, where each chromosome represents intact ancestry inherited from each parent, 2) an offspring of a backcross (BC) to the first parent (BC1) and 3) an offspring of a backcross to the second parent (BC2). Offspring within the gray box are admixed ancestries (assuming each parent was of non-admixed ancestry themselves). With each increasing admixture event, offspring can result in dramatically variable ancestry compositions.

The scientific taxonomic name of gray wolf is *Canis lupus*, where “*Canis*” is the genus and “*lupus*” the species identifier. Dogs are often referred to as *Canis familiaris* or *Canis lupus familiaris*, with the later taxonomic name indicating the dog is a subspecies of the *Canis lupus* lineage. To more broadly refer to both dogs and wolves, without specifying which species, the term “*canine*” or “*canid*” is used. Here, I use the more general term of “*canine*” to refer to a canine-like individual, deriving exclusively of the *Canis* genus.

There are three types of genetic material in vertebrates: DNA carried by the mitochondrion (referred to as mtDNA), DNA carried by the sex X/Y chromosomes, and DNA carried on the autosomes (all other non-sex chromosomes). Canines have 38 autosomes, plus a single pair of the sex chromosomes. An individual’s mtDNA is exclusively passed on by their mothers and represents the matriline. To understand more about the mother’s ancestry, one would look at the genetic composition of the mtDNA. Paternal information is only passed on from fathers to sons in the form of the Y chromosome. Females generally do not inherit a Y chromosome and thus paternal information can only be obtained in males to understand more about their father’s ancestry.

The results of the DNA testing conclusively display that the canines under question are irrefutably gray wolves.

I was contracted to review and interpret the results of multiple genetic tests conducted on buccal swabs collected from 10 canines (FCD487 case # 1 through 10) to determine their ancestry and species membership. Below, I have provided my expert interpretation regarding species assignment of the canines in question.

Summary of Embark results and interpretation. This company advertises a genetic assay of >200,000 markers. This test provides an easy visualization of results, with the most impressive and useful depiction of ancestry color coded along each of the 38 canine chromosomes. The proportion of a genome that is inferred to be derived from gray wolf is assessed using approximately 500 markers that have been previously published to be highly divergent between dogs and wolves. Ancestry is inferred from approximately 200,000 markers evenly distributed across the genome.

The chromosome schematics are fantastic and provide an incredible foundation upon which I state that it is highly unlikely that each of the animals tested is a hybrid. There

are, however, three scenarios that can explain the admixture ancestry proportions of the canines tested.

- The most likely scenario is that the canines in question come from a line of individuals extensively backcrossed to North American wolves, and carry negligible remnants of possible Northern breed ancestry in their paternal lineage,
- The second scenario is the inherent limitation of sequencing technology. Due to the recent evolutionary relationships between dogs and wolves, and with the consequences of this close relationship (predominantly in the high degree of shared genetic material), genetic tests that can absolutely 100% discriminate wolves and dogs is incredibly challenging to develop. For example, Embark reports that it is hard to discriminate dog from wolf ancestry in some cases. Although Embark collects and evaluates a large fraction of the genome, it is still not every nucleotide in the entire genome of every canine on Earth and throughout evolutionary history. As such, there are bounds and limitations for inferring canine ancestry. The admixture proportions may well indicate the limitations of our knowledge and ability to infer complex, past evolutionary events between closely related canines.
- The third scenario is that all animals are pure wolves and, due to limitations of genetics assay based in their recent shared evolutionary history, ancestry is confounded by shared genetics and the inability to test every single canine that ever existed from today, back through evolutionary time.

The chromosome schematics represent regions of the chromosome assigned to the reported breed or wolf identity. Referring back to [Figure 1](#), there is an expectation that a recently admixed individual would show a significant fraction of the genome assigned to both dog and wolf. A first or second generation backcross to wolf would still display a substantial amount of dog ancestry. Although from these tests we are not explicitly able to determine how many generations ago a hybridization event occurred (if it did), these chromosome depictions show that the overwhelming majority of the genome is derived from wolf (yellow colored chromosomal regions in the Embark report).

Each canine in question carried maternal genetic material (mtDNA) that derived exclusively from Asia, with an identifying source of either a Northern breed (e.g. Akita) but not to the exclusion of genetic material also likely of other wolf-dog crosses or wolves. This means that the mothers of the canines in question was a Northern breed, a wolf-dog cross, or a gray wolf.

Each male canine in question carried paternal genetic material (Y chromosome) that is exclusively derived from Canada, with the haplotype not previously found in the reference database of dog Y chromosomes. This means that the fathers of the canines in question was very likely a gray wolf, with very low probability that he was a dog.

The defendants additionally contracted Embark to conduct DNA testing of many of the same canines in question. I have reviewed those results and find that canines with repeated testing are concordant across both tests.

Summary of UC Davis results and interpretation. This genetic assay surveyed 38 markers, consisting of short tandem repeats (STRs) across the autosomes, seven Y-chromosome markers, two sex-linked markers, and sequence from the HV1 region of the

mtDNA. Genetic ancestry was based on analysis of the STR data, while Y-chromosome and mtDNA data were used for assessing the taxonomic affiliations of paternally- and maternally-inherited DNA, respectively.

The STR markers were genotyped in 53 reference gray wolves and 101 dogs derived of Northern breeds. This reference panel was used for testing each individual canine under question separately from the others. As a point of potential bias, it is unclear why only Northern breeds were used as the dog reference. Although phenotypes can be misleading, especially in canines, the use of Northern breeds as reference dogs are still a valid and legitimate comparison. In review of both company's tests, the level of concordant findings is promising to ensure that UC Davis's results are interpretable as is.

Parameters for the Bayesian analysis of the STR data were common and acceptable (75,000 burn-in, and 300,000 reps). As confidence intervals for assignment to each dog and wolf do not overlap, the assignment probabilities are not ambiguous and can be used to confidently infer taxonomic affiliation (i.e. dog or wolf).

As all individuals carry the matrilineal mtDNA regardless of sex, all individuals were surveyed and found to carry mtDNA derived only from wolves. However, only male individuals could be surveyed for their Y chromosome. A single canine (case #8) carried a never-before observed Y chromosome, while the rest of the males (cases #1-4, 7, and 9) carried a Y chromosome derived from wolves.

Summary. I have reviewed results from both genetic testing companies and find that both tests provide concordant and parallel results. Embark's test can more directly support the inference that the canines in question are not recently admixed nor hybrids with a non-wolf entity (coyote or dog). My interpretations are bulleted below:

- i. Nine of the 10 canines tested had a high occurrence of wolf-specific alleles, with negligible amounts of dog genetic content. This was consistent across companies and DNA marker types (autosomal, sex-linked, and paternally or maternally inherited material).
 - o Only a single canine in question contained a non-negligible proportion of dog ancestry (case #10; 67.3% gray wolf and 32.7% dog), which is possibly indicative of recent admixture with dog. Paternal Y chromosome information was not provided; however, the maternal mtDNA material is consistent with the other canines tested, indicating case #10's mother was a Northern breed, a wolf-dog cross, or a gray wolf. This individual's test results from UC Davis confirm this individual as a gray wolf, with higher wolf ancestry (85% gray wolf and 15% dog).
- ii. Based on the chromosomal composition of ancestry, there is no support to suggest that the canines in question are anything but high in wolf genomic content. They are not hybrids, and a small fraction of their genome is admixed. This level of admixed ancestry is not uncommon to find in wild gray wolves, other canines, and other species (see below sections for details).

Morphology is consistent with wolf morphology but morphology, in general, is not sufficient to assign species membership.

Though heavily relied upon as information for species designation prior to the molecular age (i.e. 1990s), morphology can be quite inconclusive at best, perhaps even misleading. Many people use the appearance of an animal to infer ancestry (e.g. deconstructing the ancestry proportions of mixed breeds), with often conflicting results when compared to genetic assays. I only provide my comments here regarding morphology due to the available of images of the canines in question.

I have also reviewed several photos of the canines in question. Below you will find my comments regarding the general appearance regarding their structure, pigmentation, and patterns.

- i. Doc 20-4 Liebman Decl. Ex B-4 Wolf Pelts: All pelts visible in this photo conform to the expected general agouti appearance of North American gray wolves. The pigmentation deposition follows expected banding patterns along hair shafts, alternating between black and yellow bands, falling within the typical coloration spectrum and patterns of gray wolves. This does not prove nor deny taxonomic assignments.
- ii. Doc 20-3 Liebman Decl. Ex B-3 Letter to USDA: I reviewed images 2.1-2.5, 2.7-2.10. All canines that appeared in these images again represent typical pelage coloration of gray wolves, although this is not mutually exclusive to coloration that can appear in a few domestic dog breeds.
- iii. Deposition Exhibits 14 and 15 Wolf pelts: All pelts visible in this photo conform to the expected general agouti appearance of North American gray wolves. The pigmentation deposition follows expected banding patterns along hair shafts, alternating between black and yellow bands, falling within the typical coloration spectrum and patterns of gray wolves. This does not prove nor deny taxonomic assignments.
- iv. Deposition Exhibits 16 and 17 Wolf: This individual has a pelage coloration typical of North American gray wolves. Further, the cranial morphometrics (size, shape) appear to be as expected: “blocky” shape with wide-set ears rounded at the top, wide rounded muzzle, square and wide check circumference. However, some dog breeds have been bred to retain this appearance, either through admixture with wolves when establishing the breed or through modern day hybridization. Further, even among the fossil record, it is challenging to clearly distinguish from morphometrics alone the taxonomic membership of canines.
- v. Deposition Exhibit 18 Wolves: The photo is dark and difficult to resolve fine details. The individual on the right displays similarities to a Spitz-type breed (e.g. husky, malamute), with Spitz breeds often sharing similar physical features with wolves.
- vi. Deposition Exhibits 19 and 20 Wolves: Darker in pigmentation, the individuals in this photo appear to have a slight narrowing of craniofacial features.
- vii. Deposition Exhibits 56 and 57: This blurry image displays an above-view of a pelt with narrow craniofacial features. However, the narrow appearance could

- be an artefact of the distance away from the pelts (no rule is provided for inferring size in the image).
- viii. Deposition Exhibit 59: The two pelts visible in this photo conform to the expected general agouti appearance of North American gray wolves (described above). The craniofacial structure appears to be wide, with rounded muzzle. This does not prove nor deny taxonomic assignments.
 - ix. Deposition Exhibit 60: The two pelts visible in this photo display the ventral view (under-side), both of which are extremely light in coloration. This “white” coloration is not uncommon among North American gray wolves. I suspect this is the ventral view of Deposition Exhibit 59.
 - x. Deposition Exhibit 61: I suspect this is the cranial view of Deposition Exhibit 59. See my comments for Deposition Exhibit 59.
 - xi. Deposition Exhibits 65 and 66: The pelt visible in this photo conform to the expected general agouti appearance of North American gray wolves (described above). The craniofacial structure appears to be wide, with rounded muzzle. This does not prove nor deny taxonomic assignments.
 - xii. Deposition Exhibits 69 and 70: The three pelts in this photo appear with light to agouti coloration, with narrow craniofacial features. However, the narrow appearance could be an artefact of the distance away from the pelts (no rule is provided for inferring size in the image).
 - xiii. Deposition Exhibit 71: Appears to be a close-up cranial image of one of the very light pelts in Deposition Exhibit 69. Ears appear to be fairly rounded.

Many of the images reviewed contain individual canines that carry many features consistent with gray wolves. However, there are some dog breeds that also contain some of those features, obtained either through directed breeding programs for those traits or through historic hybridization events with wolves. These images are not conclusive and may only be informative on rare, exceptional occasions. This is not one of those occasions.

Ancestry inference can reflect a complex history.

There are several ways in which a canine’s genome could result in a complex composition of admixed ancestry. Many of the lineages of the genus *Canis* (dogs, wolves, and coyotes) are recently diverged. Their species boundaries appear to be permeable, with inter-species breeding feasible and producing reproductively viable offspring. Consequently, each of these three lineages are expected to share a non-negligible amount of genetics, making species tests for them challenging. To have full confidence to interpret specifically the genetic results, a deep understanding of the reference panel of canids is needed.

Admixture is prolific, with ecology likely playing a more significant role than ancestry in conservation.

Our concept of a species, and the genetic foundation of such a concept, is constantly evolving in light of new genomic information and technological advances. Annually, hundreds of genomes are being scrutinized relative to conservation decisions, with many of them revealing a history of admixture and gene flow. Much of these studies are

promoting the concept that admixed ancestry is prolific, including our own human evolutionary history. The conversation is shifting from “*should* hybrids be protected?” to “*how* can we best preserve biodiversity and adaptive potential?”. Further, conservation is also being encouraged to take the perspective of the ecosystem, and the ecological function of a species. The ability to resolve ancestry is challenging, to say the least. Our genomic tools are advancing at a significant rate; yet, we understand very little about the evolutionary history of genomes and not consensus regarding the statistical methods for which confident ancestry can be inferred without a series of caveats and conditionals. The inclusion of animals with mixed ancestry is critical for species conservation; as admixture is prolific, the preservation of species must extend beyond those of “genomic purity” and include those of admixed ancestries as the perspective shifts from the level of the individual to the preservation of genomic diversity and evolutionary lineage.

Glossary.

Mitochondrial DNA (referred to as mtDNA): the DNA found within mitochondria, organelles found within each eukaryotic cell whose primary function is to convert chemical energy (from food) into a form of energy that cells use (i.e. ATP).

Short tandem repeat (referred to as STR and the plural of STRs): a microsatellite (repetitive DNA) that consists of a repeating unit of DNA anywhere from two to 13 nucleotides that repeat hundreds of times in a row on a strand of DNA. These markers show consistent Mendelian inheritance with a high mutation rate that typically increases or decreased the number of repeat units found on that strand of DNA. As such, they represent a genetic marker that is informative for estimates of genetic diversity, parentage, and population dynamics (e.g. dispersal, migration, gene flow).

Northern breed group: A group of domestic dog breeds used by kennel clubs that typically consist of Spitz-type breeds that originated in the Arctic and northern latitudes of the world. For example, this breed groups includes but is not limited to the Alaskan malamute, Alaskan husky, American eskimo, Norwegian elkhound, Akita, Chow chow, Greenland dogs, Keeshond, Samoyed, Shiba Inu, Siberian husky, [Inuit] Sled dogs, etc.

Canine: The scientific taxonomic name of gray wolf is *Canis lupus*, where “*Canis*” is the genus and “*lupus*” the species identifier. Dogs are often referred to as *Canis familiaris* or *Canis lupus familiaris*, with the later taxonomic name indicating the dog is a subspecies of the *Canis lupus* lineage. To more broadly refer to both dogs and wolves, without specifying which species, the term “*canine*” or “*canid*” is used. This term is typically used in the general sense to refer to a canine-like individual, deriving exclusively of the *Canis* genus.

Full CV of Dr. Bridgett vonHoldt.

When reviewing my publication list, first and last authorship names are the most coveted positions (as well as corresponding author) in my professional field. These authorship positions signify roles of study conception, data collection, project funding, analysis, and manuscript preparation.

Peer-reviewed publication record

- BM vonHoldt**, RY Kartzinel, CD Huber, VL Underwood, Y Zhen, K Ruegg, KE Lohmueller, TB Smith (*in review*) Growth factor gene *IGF1* is associated with bill size in an African finch (*Pyrenestes ostrinus*). *Nature Communications*.
- BM vonHoldt**, E Heppenheimer, I Janowitz-Koch, KE Brzeski, KA Cassidy, DR Stahler, JS Sinsheimer (*in revision*) Heritable aggression in a pedigreed gray wolf population is associated with neurogenic variation. *Molecular Ecology*.
- BM vonHoldt**, SS Ji, ML Aardema, DR Stahler, MAR Udell, JS Sinsheimer (2018) Activity of genes with functions in human Williams-Beuren Syndrome are impacted by mobile element insertions in the gray wolf genome. *Genome Biology & Evolution*. doi: 10.1093/gbe/evy112
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- BM vonHoldt**, J Cahill, Z Fan, I Gronau, J Robinson, JP Pollinger, B Shapiro, J Wall, RK Wayne (2016) Whole-genome sequence analysis shows that two endemic species of North American wolf are admixtures of the coyote and gray wolf. *Science Advances* 2, e1501714.
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- I Janowitz Koch, MM Clark, MJ Thompson, KA Deere-Machemer, J Wang, L Duarte, GE Gnanadesikan, EL McCoy, L Rubbi, DR Stahler, M Pellegrini, EA Ostrander, RK Wayne, JS Sinsheimer, **B vonHoldt** (2016) The concerted impact of domestication and transposon insertions on methylation patterns between dogs and gray wolves. *Molecular Ecology* 25(8), 1838-1855.
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- H Huson, **B vonHoldt**, M Rimbault, AM Byers, JA Runstadler, HG Parker, EA Ostrander (2012) Selection for breed-specific ancestry targets a mutation in the MYO9 gene associated to heat tolerance within performing Alaskan sled dogs. *Mammalian Genome* 23, 178-194.
- T Coulson, DR MacNulty, DR Stahler, **B vonHoldt**, RK Wayne, DW Smith (2011) Modeling effects of environmental change on wolf population dynamics, trait evolution and life history. *Science* 334, 1275-1278.
- D Earl, **B vonHoldt** (2011) STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetic Resources* 4 (2), 359-361.
- E Geffen, M Kam, R Hefner, P Hersteinsson, A Angerbjorn, L Dalen, E Fuglei, K Noren, JR Adams, J Vucetich, TJ Meier, LD Mech, **B vonHoldt**, DR Stahler, RK Wayne (2011) Kin encounter rate and inbreeding avoidance in canids. *Molecular Ecology* 20, 5348-5358.
- B vonHoldt**, J Pollinger, D Earl, JC Knowles, AR Boyko, H Parker, E Geffen, M Pilot, W Jedrzejewski, B Jedrzejewska, V Sidorovich, C Greco, E Randi, M Musiani, R Kays, CD Bustamante, EA Ostrander, J Novembre, RK Wayne (2011) A genome-wide perspective on the evolutionary history of enigmatic wolf-like canids. *Genome Research* 21, 1294-1305. [cover image]
- B vonHoldt**, D Stahler, EE Bangs, DW Smith, MD Jimenez, CM Mack, CC Niemeyer, J Pollinger, RK Wayne (2010) A novel assessment of population structure and gene flow in gray wolf populations of the Northern Rocky Mountains of the United States. *Molecular Ecology* 19, 4412-4427.
- AR Boyko, P Quignon, L Li, JJ Schoenebeck, JD Degenhardt, KE Lohmueller, K Zhao,

- A Brisbin, HG Parker, **B vonHoldt**, M Cargill, A Auton, A Reynolds, AG Elkhoun, M Castelano, DS Mosher, NB Sutter, GS Johnson, J Novembre, MJ Hubisz, A Siepel, RK Wayne, CD Bustamante, EA Ostrander (2010) A simple genetic architecture underlies quantitative traits in dogs. *PLoS Biology* 8(8).
- I Jankovic, **B vonHoldt**, N Rosenberg (2010) Heterozygosity of the Yellowstone wolves. *Molecular Ecology* 19, 3246-3249.
- B vonHoldt**, E Han, J Pollinger, KE Lohmueller, E Han, HG Parker, P Quignon, JD Degenhardt, AR Boyko, DA Earl, A Auton, A Reynolds, K Bryc, A Brisbin, JC Knowles, DS Mosher, TC Spady, A Elkhoun, E Geffen, M Pilot, W Jedrzejewski, C Greco, E Randi, Danika Bannasch, A Wilton, J Shearman, M Musiani, M Cargill, PG Jones, Z Qian, W Huang, ZL Ding, YP Zhang, CD Bustamante, EA Ostrander, J Novembre, RK Wayne (2010) Genome-wide SNP and haplotype analyses reveal a rich history underlying dog domestication. *Nature* 464, 898-903.
- E Cadieu, M Neff, P Quignon, K Walsh, K Chase, HG Parker, **B vonHoldt**, A Rhue, A Boyko, A Byers, A Wong, DS Mosher, AG Elkhoun, TC Spady, A Andre, KG Lark, M Cargill, CD Bustamante, RK Wayne, EA Ostrander (2009) Coat variation in domestic dogs is governed by variants in three genes. *Science* 326, 150-153.
- H Parker, **B vonHoldt**, P Quignon, EH Margulies, S Shao, DS Mosher, TC Spady, A Elkhoun, M Cargill, PG Jones, CL Maslen, GM Acland, NB Sutter, K Kuroki, CD Bustamante, RK Wayne, EA Ostrander (2009) An expressed fibroblast growth factor 4 (*fgf4*) retrogene causes breed-defining chondrodysplasia in the domestic dog. *Science* 325 (5943), 995-998.
- T Anderson, **B vonHoldt**, S Candille, M Musiani, C Greco, DR Stahler, DW Smith, B Padukasahasram, E Randi, JA Leonard, CD Bustamante, EA Ostrander, H Tang, RK Wayne, GS Barsh (2009) Molecular and evolutionary history of melanism in North American gray wolves. *Science* 323 (5919), 1339-1343. [cover image]
- B vonHoldt**, D Stahler, D Smith, DA Earl, JP Pollinger, RK Wayne (2008) The genealogy and genetic viability of the reintroduced Yellowstone grey wolves. *Molecular Ecology* 17, 252-274.
- B vonHoldt**, E Ostrander (2006) Preview: The singular history of a canine transmissible tumor. *Cell* 126, 445-447.

Popular science publication record

- B vonHoldt** and C Driscoll (2016) Origins of the dog: Genetic archaeology and molecular architecture. *In* The Domestic Dog: Its Behavior, Evolution and Interactions with People. Cambridge University Press, UK.
- B vonHoldt**, M Gray, and R Wayne (2012) Genome-wide approaches for the study of dog domestication. *In* Biodiversity in Agriculture: Domestication, Evolution, Sustainability. Cambridge University Press, UK.

Grants & Awards

National Science Foundation

- Division of Integrative Organismal Systems: Proposal Number IOS-1754503
 - Duration: 2018-2021
 - Funded: \$135,081
 - Title: Maladaptive plasticity and hypoxia signaling in high-altitude deer

mice.

- EPSCoR: Proposal Number OIA-1738597
 - Duration: 2018-2020
 - Funded to PI: \$299,999
 - Title: Mechanisms underlying transgenerational inheritance of the stress phenotype.
- EAGER: Proposal Number DEB-1257716
 - Duration: 2012-2014
 - Funded: \$124,928
 - Title: Functional testing of gene variants through transcriptome analysis of cell lines established from North American gray wolves.

Yellowstone Park Foundation

Duration: 2015

Funded: \$12,000

Title: Genome-wide genotyping efforts for pedigree and fitness-trait modeling.

Point Defiance Zoo & Aquarium Dr. Holly Reed Conservation Fund

Duration: 2015

Funded: \$14,680

Title: Pedigree analysis of genome-wide ancestry in the endangered red wolf.

American Kennel Club Canine Health Foundation

Duration: 2012

OAK Grant Number: CHF 1822

Funded: \$119,692

Title: Developing resources and exploring the role of the epigenome in canine health.

Princeton University nominee

- Johnson & Johnson Women in Science STEM²D Scholar Program in 2017
- Searle Scholars Program in 2013

Conferences & workshops

- Adaptive Epigenomics workshop: Building a bridge between animal and human research, at the University of Konstanz, Germany (2017)
- Smithsonian BioGenomics 2017 Conference (Scientific organizing committee member) (2016-2017)
- Dog10K Consortium (member) in Beijing, China 2016
- Faculty for the Conservation Genetics 5-day Workshop at the University of California, Los Angeles (2016)
- Professional Development Workshop (panelist) at Princeton University (2015, 2016)
- Species domestication Symposium Chair, Society for Molecular Biology and Evolution, in Puerto Rico (2014)
- Recent Advances in Conservation Genetics 10-day Workshop (Teaching faculty)

- Mayagüez, Puerto Rico (2015)
- Kruger National Park, South Africa (2013)

Patents & technology

Docket #17-3362 (filed with Princeton University)

- Title: Simple genomic test of canine genes associated with Williams-Beuren Syndrome could predict social behavior in domesticated dogs
- Inventors: Bridgett vonHoldt (Princeton University), Janet Sinsheimer (UCLA), and Monique Udell (OSU)

Docket #2017-644-1/17-3320 (filed with UCLA)

- Title: Using DNA methylation markers to predict the age of dogs
- Inventors: Matteo Pellegrini (UCLA), Stefan Horvath (UCLA), Bridgett vonHoldt (Princeton University), Michael Thompson (UCLA)

Scientific outreach

Princeton University Learning Laboratory (High School Internship)

- Project: Goldenrod population genetics; Students: Dominica Colavito, Katie Simons
- Project: Genetics of canine mange; Students: Amelia Krause
- Project: Camera trap analysis of African fauna; Students: Olivia Ondis

Science Education and Outreach Workshops

- Public seminar: Science on Tap at the Triumph Brewery, Princeton, NJ (2018)
- Public Lecture: 66th Annual Mendel Lecture at the St. Peter's University, Jersey City, NJ (2017)
- Guest on Princeton's WPRB radio show *These Vibes Are Too Cosmic* (2016)
- Student Conference on Conservation Science mentor at the American Museum of Natural History (2015)
- New Jersey Science Convention, Invited presentation on "*Canines can serve as excellent case studies for teaching genes, inheritance, and selection*" to 9-12th grade STEM teachers (2014)
- HHMI Invited Mentor to the University of Puerto Rico, Mayaguez; Two-week intensive mentoring and teaching program (2014). My hosts (Drs. Martinez-Cruzado and Oleksyk) organized a Departmental seminar, a bioinformatic tutorial workshop, and student meetings to discuss research and career planning.
- Animal Behavior Society Conference, Family Outreach Workshop, "*Canine Traits: Learning about genes and inheritance.*" (2014)
- Better Education for Women in Science and Engineering, "How Genes Work Together to Make a Dog", Canine Genetics and Inheritance Session (2012)
- "DNA Day", Natural Selection Session; with the Genomic Analysis and Interpretation Training Grant (2009, 2010)

Invited seminars

- Biology Department, University of Konstanz, Germany, 2017

- Richard Gilder Graduate School, American Museum of Natural History, 2017
- New Mexico State University, 2016
- American Museum of Natural History, 2016
- Sichuan University, Chengdu, China, 2016
- Chinese Academy of Sciences, Beijing, China, 2016
- Society for Molecular Biology & Evolution Satellite Meeting: Genetics of Admixed Populations, 2016
- Northeast Naturalist History Conference, 2016
- Michigan State University, Department of Ecology, Evolutionary Biology, and Behavior, 2016
- New Jersey Humanist Network, New Jersey Audubon Society, 2016
- University of Georgia, Department of Warnell School of Forestry and Natural Resources, 2015
- New York University, Department of Biology, April 2015
- Princeton University, Department of Molecular Biology, 2014
- Fordham University, Department of Biological Sciences, 2014
- Bard College, Biology Department, 2014
- Pennsylvania State University, Center for Comparative Genomics & Bioinformatics, 2013
- University of Illinois, Urbana-Champaign, Animal Science, 2012
- Dartmouth University, Department of Biological Sciences, 2012
- Princeton University, Ecology and Evolutionary Biology, 2011
- UCLA Genome Analysis and Interpretation Training Program, 2011
- UCLA 9th Annual Symposium, Center for Society and Genetics, 2011
- Cornell University, College of Veterinary Medicine, 2011

Teaching experience

Courses

Princeton University, Dept. of Ecology and Evolutionary Biology

- | | |
|--|---------------------|
| • Seminar: Polyphenism, EEB524 | <i>Spring 2016</i> |
| • Junior Tutorial: Evolution | <i>Fall 2014</i> |
| • Seminar: Pigmentation Genetics, EEB524 | <i>Spring 2014</i> |
| • Fundamentals EEB504 | <i>2013-2016</i> |
| • Evolution EEB309 | <i>2013-present</i> |

UCLA; Dept. of Ecology and Evolutionary Biology

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| • Special Topics Seminar: Epigenomics EEB297 | <i>Winter 2012</i> |
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University activities

Princeton University Ecology & Evolutionary Biology

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| • Member, Departmental Website Committee | <i>2017</i> |
| • Member, Graduate Admissions Committee | <i>2013-present</i> |
| • Member, Faculty Search Committee | <i>2014-present</i> |

Princeton Institute for Computational Science & Engineering

- Member, Computational Science & Engineering Curriculum *2018-present*

Professional activities

Associations

American Genetic Association, Council member *Jan 2016-Dec 2018*

Associate Editor

- Molecular Ecology Resources *2017-present*
- Molecular Ecology *2017-present*
- Ecology Letters *2016-present*
- Journal of Heredity *2015-present*
- Molecular Ecology, Special Issue on Epigenetics *2014-2015*

Advisees

Post-doctoral Researchers

- Kristin Brzeski 2016-2017
- Rebecca Kartzinel 2016-2017
- Linda Rutledge 2015-2016
- Ilana Janowitz 2014-2016
- Kerry Machemer 2013-2016

Graduate Advisees

- Alexandria DeCandia, Ph.D. student 2015-present
- Elizabeth Heppenheimer, Ph.D. student 2014-present

Graduate Ph.D. Committee

- Jacqueline Robinson, UCLA EEB 2016-2017
- Malavika Rajeev, Princeton University EEB 2016-present
- Lu Yang, Princeton University EEB 2015-present
- Maria Cavedon, University of Calgary 2015-present
- Molly Schumer, Princeton University EEB 2013-2016

Undergraduate Senior Thesis Advisees

(* thesis is a manuscript in prep/published)

- Class of 2019: Maddie Offstein, Robbie von der Schmidt, Mikaela Walkup, Riley Wilkinson
- Class of 2018: Julian Goldman, Tabitha Lumour-Mensah, Larkin Papa
- Class of 2017: Catherine Cao, Rohan Hylton, Quin Pompei
- Class of 2016: Daniela Cosio*, Carly Jackson, Jordan Lubkeman, Emily Shuldiner*, Samantha Wu
- Class of 2015: Thomas Kroshus, Karlos Bledsoe
- Class of 2014: Rachelle Mariano*, Gitanjali Gnanadesikan*, Eskender McCoy*

Visiting Student Research Collaborator

(* undergraduate as coauthor on a manuscript in prep/published)

- Fabricio Silva Garcez, Pontifical Catholic University of Rio Grande do Sul, Brazil
- Constance Braz* (Undergraduate), Virginia State University
- Deja Rogers* (Undergraduate), Virginia State University
- Keana Johnson* (Undergraduate), Virginia State University
- Gayle Pedersen, University of Pretoria, South Africa
- Jenni Harmoinen, University of Oulu, Finland
- Yashira Afanador-Hernandez, University of Puerto Rico, Mayaguez

Affiliations

Princeton University

- Princeton Institute for Computational Science & Engineering
- Molecular Biology Department, Associated Faculty
- Quantitative Computational Biology, Associated Faculty

External

- American Museum of Natural History, NY; Visiting scientist (2017-present)

Media coverage

B vonHoldt, *et al.* (2017) Structural variants underlie hyper-sociability: A novel theory of dog domestication. *Science Advances* 3, e1700398.

- Radio Times on Philadelphia WHYY, <http://why.org/cms/radiotimes/2017/08/01/dog-evolution/>
- BBC, <http://www.bbc.com/news/science-environment-40655634>
- Princeton homepage, <https://www.princeton.edu/news/2017/07/19/researchers-identify-common-underlying-genetic-basis-social-behavior-dogs-and>
- National Geographic, <http://news.nationalgeographic.com/2017/07/dogs-breeds-pets-wolves-evolution/>
- Science News, <http://www.sciencemag.org/news/2017/07/what-makes-dogs-so-friendly-study-finds-genetic-link-super-outgoing-people>
- The New York Times, <https://www.nytimes.com/2017/07/19/science/dogs-genes-sociability.html?smid=pl-share>
- Smithsonian, <http://www.smithsonianmag.com/smart-news/your-dog-loves-you-because-genes-180964110/>
- New Scientist, <https://www.newscientist.com/article/2141308-first-dogs-may-have-been-extremely-sociable-wolves/>
- Inside Science, <https://www.insidescience.org/news/rare-human-syndrome-may-explain-why-dogs-are-so-friendly>
- The Verge, <https://www.theverge.com/2017/7/19/16000172/dogs-wolves-canines-genetics-evolution-domestication-love-best-friends>
- phys.org, <https://phys.org/news/2017-07-dogs-doting-companions-genes.html>
- Inverse Science, <https://www.inverse.com/article/34377-dogs-friendly-loyal-good-boy-genetics>

BM vonHoldt, J Cahill, Z Fan, *et al.* (2016) Whole-genome sequence analysis shows that two endemic species of North American wolf are admixtures of the coyote and gray wolf. *Science Advances* 2, e1501714.

- TheBrainScoop video, <https://youtu.be/oA-QINoEEwQ>
- The New York Times by C Zimmer.
<http://www.nytimes.com/2016/07/28/science/red-eastern-gray-wolves.html>
- Science Magazine, <http://www.sciencemag.org/news/2016/07/how-do-you-save-wolf-s-not-really-wolf>
- New Scientist, <https://www.newscientist.com/article/2099192-red-wolf-may-lose-endangered-status-because-its-just-a-hybrid/>
- Science Daily,
<https://www.sciencedaily.com/releases/2016/07/160727150802.htm>
- phys.org, <http://phys.org/news/2016-07-gray-wolf-endangered-species-genomic.html>
- ScienceNews, <https://www.sciencenews.org/article/distinctions-blur-between-wolf-species>
- The Wildlife Society, <http://wildlife.org/supposed-wolf-species-may-actually-be-hybrids/>
- [seeker.com](http://www.seeker.com), <http://www.seeker.com/endangered-species-act-dilemma-protect-mutts-or-just-purebreds-1946671581.html>
- The Molecular Ecologist.
<http://www.molecular ecologist.com/2016/05/disentangling-the-wolf-coyote-admixture-through-an-ancestry-based-approach/>

B vonHoldt, E Han, J Pollinger, *et al.* (2010) Genome-wide SNP and haplotype analyses reveal a rich history underlying dog domestication. *Nature* 464, 898-903. National Geographic Special: *And Man Created Dog* (aired August 2010)

- The New York Times by N Wade,
<http://www.nytimes.com/2010/03/18/science/18dogs.html>
- The Colbert Report, <http://www.cc.com/video-clips/9cthmz/the-colbert-report-middle-eastern-dogs>
- Science Magazine New Focus: Morell (2008) Wolves at the door of a more dangerous world. *Science* 319(5865), 890-892.



Bridgett vonHoldt